Organocatalysis

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A Practical Aryl Unit for Azlactone Dynamic Kinetic Resolution: Orthogonally Protected Products and A Ligation-Inspired Coupling Process**

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Abstract: *The first strategy for bringing about enantioselective azlactone dynamic kinetic resolution to generate orthogonally protected amino acids has been developed. In the presence of a C_2-symmetric squaramide-based catalyst, benzyl alcohol reacts with novel yet readily prepared tetrachloroisopropoxycarbonyl-substituted azlactones to generate trapped phthalimide products of significant synthetic interest with excellent enantiocontrol. These materials are masked amino acids which are demonstrably orthogonally protected: cleavage of the phthalimide can be achieved in the presence of the ester and vice versa. This process could be utilized to bring about a highly stereoselective ligation-type coupling of protected serines (at stoichiometric loadings) with racemic azlactones derived from both natural and abiotic amino acids. After deprotection, a subsequent base-mediated O→N acyl transfer occurs to form a dipeptide.*

The organocatalytic dynamic kinetic resolution (DKR) of azlactones ($pK_a \approx 9$)[1] by alcoholysis (Figure 1a) has been extensively investigated over the last ten years.[2–4] This focus has resulted in a number of bifunctional,[5] Brønsted basic/nucleophilic,[6] and Brønsted acidic[7] catalytic systems capable of promoting the highly efficient and enantioselective addition of low-molecular-weight alcohols to azlactones under mild reaction conditions.[8–10] These reactions provide easy access to bis(protect)ed amino acids of potential importance: for instance Song et al.[5g] have demonstrated the synthesis of a range of natural and unnatural protected amino acids of the general type **2** with uniformly high enantiocontrol starting from racemic azlactones (**1**) using the squaramide-based bis(dihydrocinchona) alkaloid **3a** (Figure 1b). However, all the current efficient organocatalytic approaches to azlactone DKR require aryl azlactone substrates such as **1**. These substrates generate (upon ring opening) the corresponding N-benzoyl-protected amino acids, the hydrolysis of which is incompatible with the retention of the ester protecting group (e.g. Song has prepared both native[5i] and α-deuterated[11] analogues of the herbicide[12] nonproteogenic amino acid *m*-tyrosine **5** from **4** using HBr-mediated hydrolysis of both functionalities and the methoxy group). Although this is not problematic if the practitioner requires the unprotected amino acid as the product, this lack of protecting group orthogonally represents a serious limitation regarding further use of the products in peptide synthesis. When using the current technology one is obliged to (often inefficiently) remove both protecting groups simultaneously (i.e., back to the free amino acid) under harsh reaction conditions, which are necessary for benzoyl amide hydrolysis to occur, and then reprotect the amino acid selectively for subsequent use in coupling chemistry. A method for the direct synthesis of orthogonally protected amino acid products by DKR would therefore significantly increase the general utility of the methodology. A second noteworthy aspect of the current state of the art is the

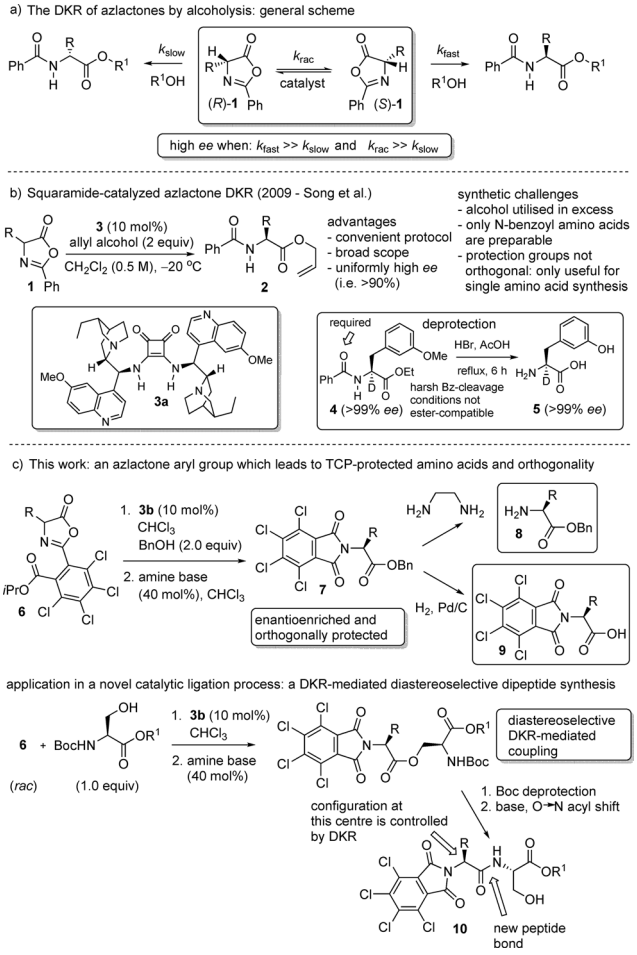

Figure 1. The DKR of azlactones by alcoholysis.

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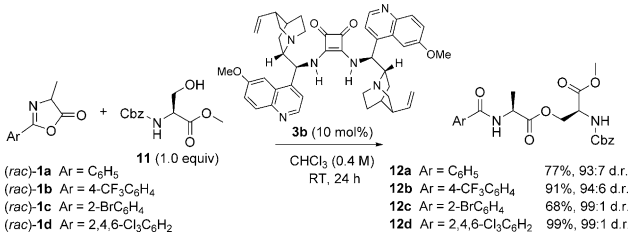
ubiquitous use of relatively unfunctionalized alcohol nucleophiles as coupling partners (i.e., alcohols with no other hydrogen-bond donating/accepting functionality).

Herein we report a solution to these challenges through the introduction of a new strategy involving the use of the modified aryl azlactones **6** (readily prepared from racemic amino acids) which upon reaction with alcohols in the presence of **3b** (Figure 1c; for structure see Scheme 1), now lead to orthogonally protected *N*-tetrachlorophthalimido amino acid benzyl esters (**7**), which can be cleanly deprotected to give either the amine **8** or the acid **9** under mild reaction conditions, with outstanding enantioselectivity in one pot. We also demonstrate the use of this strategy in a novel ligation-type process involving azlactone DKR using a serine-based alcohol. This strategy allows subsequent deprotection and O→N acyl transfer (reminiscent of native chemical ligation)[13,14] to form a dipeptide, where one of the constituent amino acids (variable) has been dynamically resolved by using the other as a nucleophile (**10**; Figure 1c).

Our investigation began with an examination of the feasibility of carrying out organocatalytic DKR using protected serine-based nucleophiles. We were also bound by considerations of a practical nature. In previous literature studies an excess of (simpler) alcohols is always used (1.2–10 equiv) and would not be feasible in a coupling methodology involving complex components. Initial results were encouraging, and after extensive preliminary studies we arrived at reaction conditions under which stoichiometric amounts of the protected serine **11** could be added to the *N*-benzoyl alanine derived racemic azlactone **1a** to yield the O-coupled product **12a** (in which the alanine unit had been resolved by addition of the serine) in a yield and with diastereocontrol which was high, but insufficient for practical coupling (Scheme 1). Despite considerable experimentation

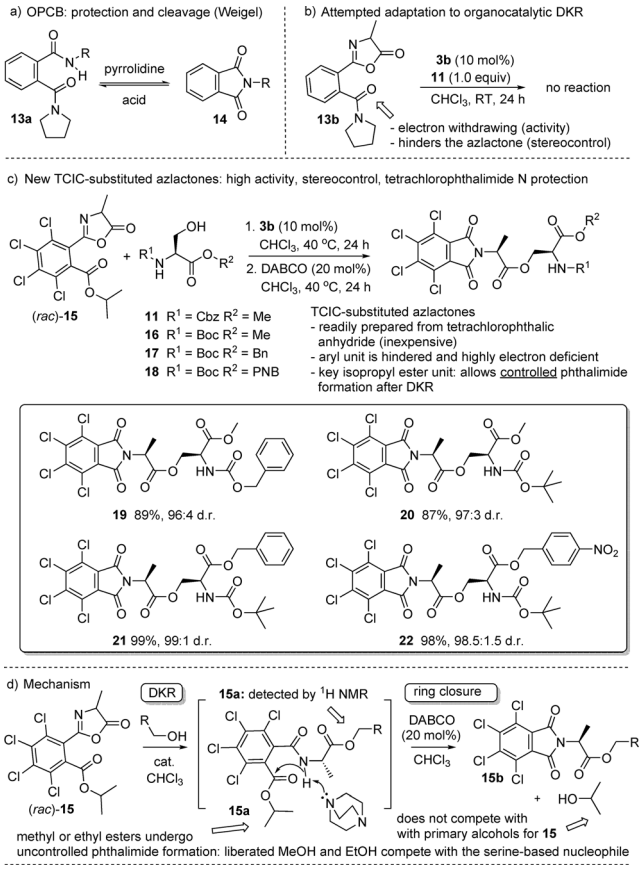


Scheme 1. The importance of hindered, electron-deficient aryl substituents in diastereoselective azlactone DKR using **11**.

aimed at developing a superior catalyst, the C_2-symmetric squaramide-based amine **3b** proved optimal. Further study led to the finding that electron-withdrawing groups and steric bulk close to the azlactone ring improved product yield and diastereoselectivity, respectively, while the combination of both in one substrate (**1d**) resulted in near-perfect efficacy and control.

Thus we were aware that efficient and diastereoselective organocatalytic azlactone DKR using serine nucleophiles was possible, but the challenges associated with product protection (i.e., orthogonality) and one-pot O→N acyl transfer remained. It was clear that the development of a new azlactone aryl substituent (destined to become the N-terminus protecting group in the product) was necessary. Specifically, we recognized that this unit must: A) be electron-deficient enough to activate the azlactone towards attack by a functionalized, nontrivial alcohol (e.g. a protected serine); B) be hindered enough to bring about near perfect diastereocontrol in the presence of catalyst **3b**; C) result in the formation of a stable N-protecting group after azlactone DKR which could be easily cleaved under mild reaction conditions in the presence of unhindered ester functionality, and would be inert to standard peptide deprotection conditions (e.g. hydrogenolysis/acidic or basic ester hydrolysis etc.).

A search of the literature did not yield a suitable moiety. We were intrigued however, by a report from 1991 from Lilly Research Laboratories,[15] in which they disclosed the utility of *o*-pyrrolidinocarbonylbenzamide (OPCB) protecting groups. These groups (e.g. **13a**; Scheme 2a) can be reversibly



Scheme 2. The design of TCIC-substituted azlactones. DABCO = 1,4-diazabicyclo[2.2.2]-octane.

converted into phthalimides (**14**). We considered the possibility that such a strategy could be applicable to organocatalytic DKR, and promptly synthesized the azlactone **13b** (Scheme 2b). This substrate incorporates the *o*-pyrrolidinocarbonyl moiety, which we hoped would provide the steric bulk and electron-withdrawing power necessary for efficient and selective DKR, but which could also be readily trans-

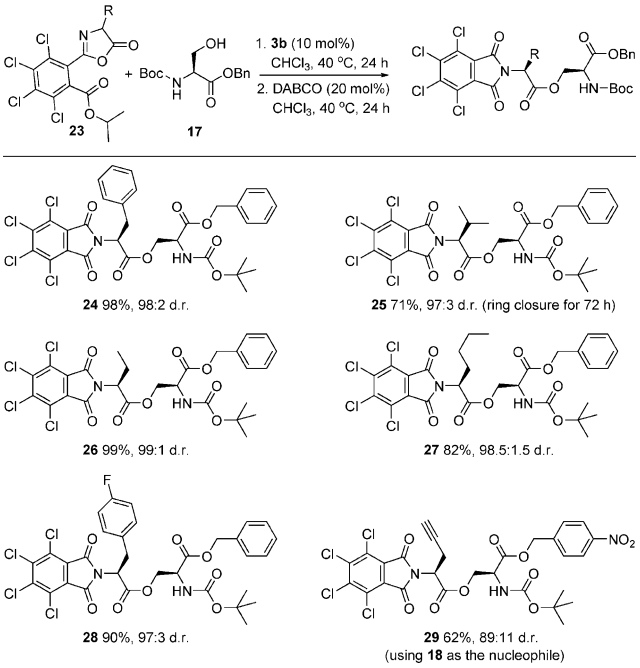
formed into an N-phthalimido-protected amino acid ester. Disappointingly, **13b** proved inert under the optimal DKR conditions.

We therefore rendered the OPCB protecting group strategy more amenable to organocatalytic DKR through the design of a novel class of tetrachloroisopropoxycarbonyl (TCIC)-substituted azlactones (e.g. (*rac*)-**15**, Scheme 2c). The chlorine atoms both improve the electrophilicity of the azlactone and also augment steric bulk at the point of attachment to the heterocycle to ensure high diastereocontrol. The exchange of the pyrrolidinamide for an isopropylester is also a key modification. The ester enhances the electrophilicity of the substrate further, and facilitates phthalimide formation by ring-closure after DKR has taken place.

Gratifyingly, the reaction of the TCIC-substituted alanine-derived azlactone (*rac*)-**15** with a range of C- and N-protected serines (**11** and **16**–**18**) led to the formation of the N-phthalimido amino acid esters **19**–**22** in good to excellent yields and excellent to outstanding diastereocontrol (Scheme 2c). After DKR had taken place, the addition of a catalytic loading of DABCO ensured the clean and efficient one-pot phthalimide formation. It is noteworthy that esters which are cleavable under very different conditions are tolerated (i.e. methyl, benzyl, and *p*-nitrobenzoyl[16] variants) as are both Boc and Cbz protection of the amino functionality. Larger protecting groups bring about very high levels of diastereocontrol.

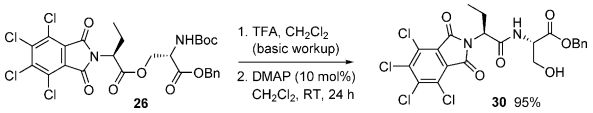
From a mechanistic standpoint (Scheme 2d), it is clear that the catalytic addition of the alcohol to (*rac*)-**15** leads to the ring-opened adduct **15a**, which was identified by ^{1}H NMR spectroscopic analysis. In the absence of DABCO some ring closure (presumably catalyzed by **3b**) occurs, however addition of the second amine catalyst leads to complete ring closure to the phthalimide **15b**. Here the requirement for the isopropylester moiety is apparent: when analogues of (*rac*)-**15** incorporating simpler methyl or ethyl esters were used, the methanol or ethanol (respectively) liberated during phthalimide-formation, catalyzed by **3b**, competed effectively with the protected serine for **15**, thus leading to the formation of some phthalimide methyl or ethyl ester products (i.e. **15b**, R = H or Me). Use of an isopropylester results in the liberation of isopropyl alcohol, which is too hindered to participate in azlactone alcoholysis, thus leading to products derived from the addition of **11** and **16**–**18** to **15** only.

We were next keen to explore the scope of the methodology. Accordingly, we reacted **17** with a range of amino-acid-derived TCIC-substituted azlactones of the general structure **23** in the presence of **3b**, followed by one-pot DABCO-mediated ring closure as before (Scheme 3). This sequence allowed the synthesis of the phenylalanine- and valine-derived O-acyl serines **24** and **25** in good to excellent yield and with excellent diastereocontrol.[17] Interestingly, in the case of the more hindered valine-derived **25**, ring closure to form the phthalimide was the slower reaction. The process was also applied to the synthesis of analogues derived from abiotic amino acids and α-ethyl- and α-*n*-butyl-substituted analogues (**26** and **27**, respectively) could be prepared with excellent stereochemical fidelity, as could the *p*-fluorophenylalanine (an important artificial amino acid chemical probe)[18] derivative **28**. A protected serine ester derivative of the expensive[19] cystathionine γ-lyase inhibitor L-propargyl glycine was also synthesized (**29**),[20] however diastereocontrol and product yields were lower (albeit still appreciable) than was the case with **24**–**28**. It is noteworthy that the major diastereomer of **29** could be readily separated from the minor by flash chromatography in an overall yield 53%.



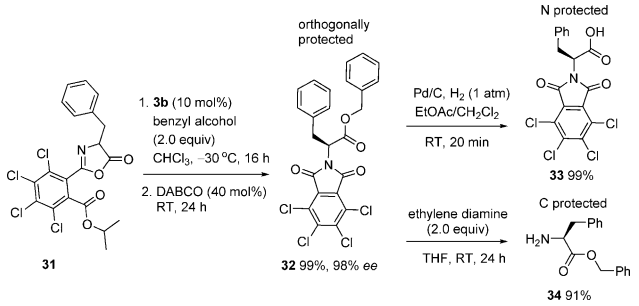
Scheme 3. Substrate scope: Natural/unnatural amino acid derivatives. Boc = *tert*-butoxycarbonyl, PNB = *para*-nitrobenzyl.

To demonstrate the potential of these materials to serve as precursors to dipeptides, **26** was first treated with trifluoroacetic acid to cleave the Boc group (Scheme 4). Removal of the volatiles in vacuo and basic workup allowed a subsequent and clean DMAP-mediated[21] O→N acyl transfer reaction to occur to yield **30** (which is orthogonally protected at the C- and N-termini) in excellent yield.

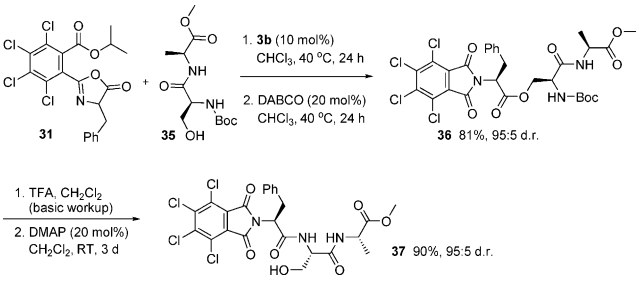


Scheme 4. Boc removal for **26** followed by O→N acyl transfer. DMAP = 4-(*N*,*N*-dimethylamino)pyridine, TFA = trifluoroacetic acid.

We wished to confirm that the products were demonstrably orthogonally protected. The azlactone **31** was prepared and exposed to benzyl alcohol in the presence of the catalyst **3b** at −30 °C (Scheme 5). This reaction resulted in the formation of **32** in near quantitative yield and 98% enantiomeric excess. It is noteworthy that product enantiomeric excess is higher than would be expected by using the azlactone **1**. The phthalimide ester **32** could be selectively deprotected. Exposure to hydrogenolysis conditions afforded the acid **33** in 99% yield, while treatment with ethylene



Scheme 5. Use of a TCIC-substituted azlactone to generate an orthogonally protected amino acid. THF = tetrahydrofuran.



Scheme 6. A DKR-peptide coupling process.

diamine allowed the cleavage of the phthalimide unit to yield the amine **34** in excellent yield.[22]

Finally, we employed a more complex nucleophile than a simple serine derivative in the coupling process (Scheme 6). The dipeptide **35** was reacted with the TCIC-substituted azlactone **31** in the presence of **3b**. The expected O-acyl adduct **36** was formed in good yield and with excellent diastereocontrol. Boc-deprotection and O→N acyl transfer subsequently generated the tripeptide **37**.

In summary, a new TCIC aryl unit has been developed for use in organocatalytic azlactone DKR chemistry. The moiety has a dual purpose. Firstly, it confers the requisite steric and electronic characteristics onto the azlactone to facilitate smooth and highly enantioselective alcoholysis reactions, and secondly, as the azlactone opens upon reaction with the alcohol it is trapped by the TCIC-pendant ester functionality to form a phthalimide ring, thereby creating an orthogonally N- and C-protected amino acid. It was also demonstrated that the process could be utilized to bring about a highly stereoselective ligation-type coupling of protected serines with racemic TCIC-substituted azlactones derived from both natural and abiotic amino acids. Following deprotection, base-mediated O→N acyl transfer occurs to form a dipeptide.

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[19] 250 mg of L-propargyl glycine currently costs € 433 (Sigma–Aldrich).
[20] In this instance the PNB ester was chosen as the serine carboxylate protecting group because of the incompatibility of benzyl deprotection with an alkyne-containing residue.
[21] In the absence of DMAP the acyl-transfer reaction proceeds slowly.
[22] Both processes could be carried out without significant loss of product enantiomeric excess. Deprotection of the benzoyl ester by hydrogenolysis must be carefully carried out to avoid loss of enantiomeric excess of greater than 0.5%. No racemization upon cleavage of the phthalimide was observed (see the Supporting Information for details).

